CARBONIC ANHYDRASE IN MOLLUSCS[1]

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The catalytic action of carbonic anhydrase in the hydration of carbon dioxide suggests the possible role of this enzyme in the deposition of carbonate in shell (Meldrum and Roughton, 1933). This relationship has received consideration with respect to shell formation in birds in which it has been found that the shell-forming tissues contain carbonic anhydrase and inhibitors of that enzyme cause deficient shell formation (Common, 1941; Benesch, Barron and Mawson, 1944; Gutawska and Pozzani, 1945).

In molluscs the $CaCO_3$ of the shell is presumably formed first in mantle tissue with at least a portion of the carbonate having its origin in metabolic carbon dioxide (Robertson, 1941). The influence of carbonic anhydrase on the conversion of carbon dioxide to carbonate in molluscs has not been studied experimentally, though the enzyme has been reported in mantle tissue of a few pelecypods (Florkin and de Marchin, 1941; Maetz, 1946) as well as in other tissues of molluscs (van Goor, 1937; Sobotka and Kann, 1941). As an approach to this problem the carbonic anhydrase content of mantle tissues has been examined in 20 species of molluscs.

Methods

Tissues were blotted, weighed and ground thoroughly with a closely fitting mortar and pestle. Distilled water equivalent to 50 times the tissue weight was added and the suspension extracted 12 to 24 hours below 5° C. (Ferguson et al., 1937). Body fluids underwent similar storage without dilution. Extracted suspensions were agitated vigorously and pipetted directly without previous centrifugation. In one set of experiments tissues were blotted, placed in vials and kept frozen for several days. They were then extracted as previously described. Enzyme values for these tissues were similar to those for freshly extracted material.

The hydration of CO_2 in mantle tissue undoubtedly occurs in the presence of both carbonate and bicarbonate; and the colorimetric method (Brinkman, 1933; Leiner, 1938) used in the present study in which CO_2 is added to a carbonate-bicarbonate solution is appropriate for the estimation of carbonic anhydrase activity in this tissue. However, the values so obtained will include the specific action of the buffer and indicator on the enzyme (Roughton and Booth, 1946). The endpoint was determined by means of a comparison tube containing phenol red buffered at pH 7.35, a value suitable both from the standpoint of rapidity of color change and rate of enzyme action. The activity of tissue extracts was measured at a final concentration of 1 : 500 and body fluids at 1 : 10. Two types of controls were used. In one the time required for reading the endpoint was determined with distilled water substituted for the extract, and in the other a portion of the extract was heated in

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TABLE I

Species	Ratio I: $\frac{\text{No extract}}{\text{Extract}}$	Ratio II: $\frac{\text{Heated extract}}{\text{Extract}}$
Pelecypoda:		
Atrina rigida		
mantle edge	0.8; 1.0; 0.9; 1.0; 1.0	1.1; 1.1; — — —
mantle without edge	0.7; 1.0; 0.9; 1.0; 0.9	1.0; 1.1; — — —
pericardial fluid	0.6; 0.9; 0.7; 0.6; 0.9*	1.2; 1.1; — — —
gill	2.8; —; 2.2; 3.4; —	4.2; —; — — —
adductor muscle	—; 0.8; 1.5; 1.2; —	—; 0.6; — — —
Venus mercenaria		
mantle edge	2.6; 4.8; 2.5;	3.4; 7.2;
mantle without edge	3.3; —; 2.6; 4.1	4.8; —
mantle cavity fluid	0.8; 1.4; 2.0;	1.5; 3.5;
Ostrea virginica		
whole mantle	4.9; 1.5; 1.8; 3.6;	7.2; 2.2
general body fluid	7.1; 2.2; —; —;	
gill	2.8; —; 5.5; —;	3.7;
adductor muscle	—; —; 1.0; —;	
Macrocallista nimbosa		
mantle edge	1.2; 1.0; 1.5; 1.9	2.0; 1.4;
mantle without edge	1.8; 2.1; 1.6;	3.0; 3.1;
mantle cavity fluid	0.6;	1.5;
gill	5.0	
adductor muscle	1.2;	
Dosinia discus		
mantle edge	1.1; 1.9; 2.1;	1.4;
mantle without edge	2.0; 2.3; 2.5;	3.1;
body fluid	1.5; 14.0*; 1.1*;	3.9;
adductor muscle	10.0; 6.4	—; —; —; 4.0
Elliptio complanatus		
mantle edge	2.5; 2.0;	2.6; 2.1;
mantle without edge	2.8; 2.5;	3.0; 2.6;
mantle cavity fluid	4.7; 1.7;	5.1; 2.0;
adductor muscle	3.0; 3.7;	2.4; 4.1;
Tagelus gibbus		
mantle edge	2.7; 2.1;	
mantle without edge	3.4; 4.2;	
mantle cavity fluid	2.7; 1.6;	
gill	5.0;	
Pecten irradians		
whole mantle	2.5; —; 2.0	
mantle edge	5.2; 2.4; —	
pericardial fluid	—; —; 1.4	
Divaricella quadrisulcata		
whole mantle*	3.2	
Ensis directis		
mantle edge	3.4	
mantle without edge	8.1	
Modiolus tulipus		
whole mantle	2.0	
Cardium muricatum		
whole mantle	4.3	

TABLE I—*Continued*

Species	Ratio I: $\frac{\text{No extract}}{\text{Extract}}$	Ratio II: $\frac{\text{Heated extract}}{\text{Extract}}$
Gastropoda:		
Busycon carica		
whole mantle	1.2; 0.8; 1.6; 1.1; 3.0; 2.1	1.7
blood	0.6; —; 0.8; 0.6;	1.9
gill	1.8;	
Viviparus japonicus		
whole mantle	1.4; 1.6	1.9; 2.2
mantle cavity fluid	0.6; 0.9	—; 1.0
Crepidula fornicata		
whole mantle	0.4; 0.6; 1.2	
mantle cavity fluid	0.9;	
Fasciolaria distans		
whole mantle	2.4; 5.1;	
blood	2.3;	
Anomia simplex		
whole mantle*	3.6	
Polynices duplicata		
whole mantle	2.7	
Sinum perspectivum		
whole mantle	2.8	
Diodora alternata		
whole mantle	2.0	

* Sample composite of tissues from several individuals.

boiling water for 5 minutes. The activity is expressed as the ratio of the time of reaction in the absence and presence of the extract and also as the ratio of time of reaction for heated and unheated extracts. Duplicate determinations were made in a large proportion of the cases and the average taken.

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RESULTS

Each figure in Table I represents the relative catalytic action of a single sample. Tissues from a particular individual are grouped in columns. In those cases in which both ratios were determined for the tissue the figures stand in corresponding positions under the two ratio headings.

It is evident that most of the tissues examined catalyzed the hydration of CO_2. Considered from the standpoint of Ratio I the outstanding exceptions are certain tissues of *Atrina rigida, Macrocallista nimbosa, Busycon carica, Crepidula fornicata,* and *Viviparus japonicus.* However, several of these instances of the absence of catalysis are only apparent as shown by comparing heated and unheated extracts (Ratio II). Of the individuals examined, carbonic anhydrase activity under the conditions of measurement is negligible or absent only in the mantle and pericardial fluid of *Atrina rigida,* the mantle-shell cavity fluid of *Viviparus japonicus,* and perhaps also in the mantle and mantle-shell cavity fluid of *Crepidula fornicata.*

The higher values for Ratio II result from the slowing of the uncatalyzed reaction by the heated extract and are probably due to the presence of carbonate and other substances.[2] Figures given by Maetz (1946) show an analogous effect. Because of this effect the values (Ratio I) of all tissues for which data on heated extracts are not available may be considered minimal except for muscle. The four adductor muscles examined showed an opposite effect and one which would be expected with acid production.

Carbonic anhydrase activity was demonstrated in the body fluids of nearly all forms studied and in the blood of two species of gastropods. Florkin (1935) and van Goor (1937) investigated blood of a large number of species and found no evidence of the enzyme. This may have been the result of the high dilutions employed.

Discussion

The presence of carbonic anhydrase in the mantle tissues of most of the molluscs examined indicates the possibility that this enzyme plays a part in the formation of carbonate. Both the mantle edge and the body of the mantle contain the enzyme and both are concerned with the deposition of carbonate (Robertson, 1941). Of course the presence of the enzyme cannot be taken as evidence that normal shell formation is dependent upon its activity, and its presence in other tissues suggests additional functions. The negligible activity in *Atrina rigida* and *Crepidula fornicata* suggests that certain forms may, in fact, lay down shell in its absence. In this connection a study of the rate of shell formation in the presence of inhibitors would be suggestive, provided the inhibitors did not interfere with the growth of other structures. The catalytic action of carbonic anhydrase may be expected to assume increased importance as the temperature is decreased, especially in forms such as the oyster in which shell formation proceeds during hibernation at temperatures below 4° C. (Galtsoff, 1934).

Summary

Carbonic anhydrase has been demonstrated in the mantle tissues and body fluids of most of 12 species of pelecypods and 8 species of gastropods. Its presence suggests the possibility of importance in shell formation in most species but negligible activity in some indicates they may deposit shell in its absence.

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[2] This effect is probably complex and may involve: (1) a change in buffer action, (2) the effect of specific substances on the enzyme, (3) an alteration of the initial pH, and (4) modification of the properties of the extract in a manner other than in destruction of the enzyme.

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